

Appendix 4: Elements of the Visual Learning Test

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Experimental maze tests have been developed to test whether a pesticide compound can disorientate foragers. Orientation performance of bees in a complex maze relies on associative learning between a visual mark and a reward of sugar solution.

The colony is maintained in an outdoor flight cage covered with an insect-proof cloth. The maze consisted of a matrix of 4 rows × 5 columns of identical cubic boxes, each side of the box measuring 30 cm; each wall has a 4 cm diameter hole in its center through which bees can move to the adjacent box (Zhang et al., 1996). The boxes are made of white opaque Plexiglas, and a metallic screen (3 × 3 mm mesh) covers the maze.

Bees fly through a sequence of boxes to reach a feeder containing a reward of sugar solution. The path through the maze spans nine boxes, including three decision boxes and six nondecision boxes. A nondecision box has two holes, each in a different wall; one hole where the bee is to enter and another hole through which the bee is expected to leave. A decision box has three holes, each in a different wall. One hole is where the bee enters and the bee is then expected to choose between the other two holes.

During conditioning, bees are collectively trained to associate a mark (designating the correct hole/path) with food. To achieve this, a mark is fixed in front of the correct hole/path as well as the sucrose solution feeder outside the maze for 1 hour. For an additional hour, the feeder is placed in the first box of the path for about 30 minutes, then in the second box of the path for the next 30 minutes, then in the third box for next 30 minutes and so on. The feeder is then moved to the fifth box for about 20 minutes. Finally, the feeder is placed at the end of the path (Figure A4.1) in the reward box. Several conditioning periods (3–5) are necessary to train a sufficient number of bees. After the bees have found the food (reward) and have fed, the bees are captured on the sugar syrup feeder and are then placed in rearing cages equipped with a water supply and a sugar syrup feeder (50% w/w). The bees are put back into laboratory and kept at a temperature of $25 \pm 2^\circ\text{C}$ in artificial light while they are labeled with colored and numbered tags.

For oral delivery of the test compound, the treatment chemical is added to a sucrose solution (50% w/w). The effect of the treatment solution on performance is then compared with that of an untreated sucrose solution. After 1.5–2 hours of starvation period, each group of tagged foragers receives a volume of the treated sucrose or the control sucrose solution, during daylight and at $25 \pm 2^\circ\text{C}$. The volumes are adjusted for a consumption of syrup estimated to be approximately 10 μL per bee. After complete consumption of the sugar solution, a new starvation period of about 2 hours is initiated. The bees are then provided with an untreated sugar solution *ad libitum* and released to a hive.

After conditioning, the capacity of an individual bee to negotiate a path through the maze is tested. An observer notes the number of correct and incorrect decisions, and then number of turns back. During retrieval

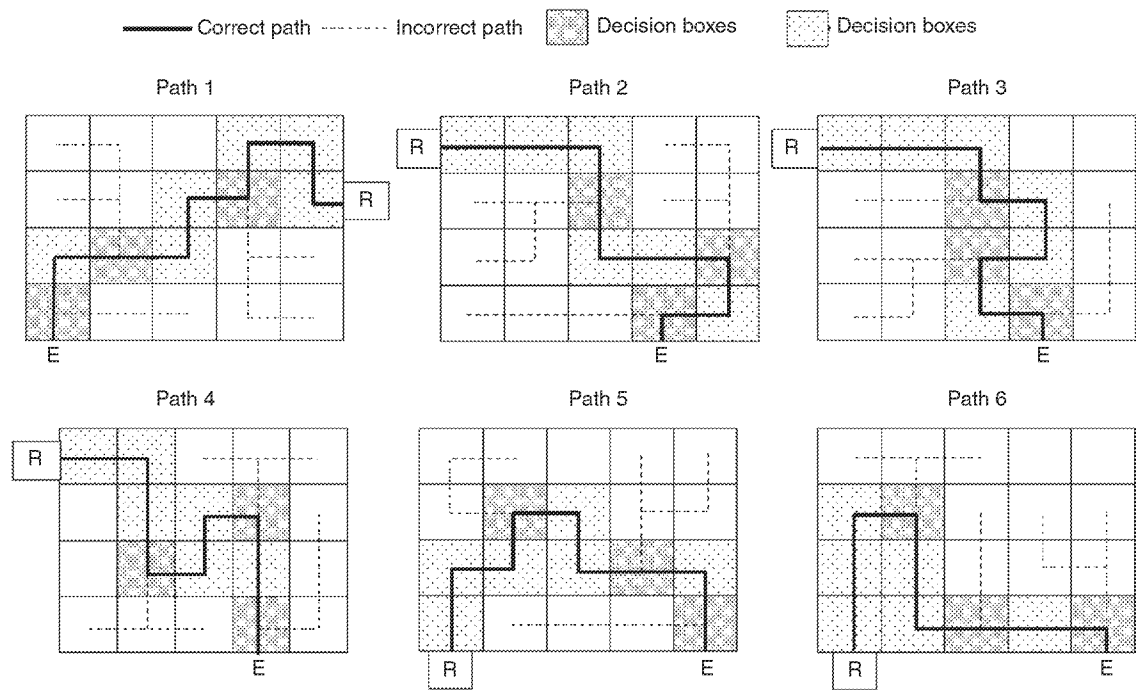


FIGURE A4.1 Maze paths used before, during, and after treatment. Path 1 is used for the conditioning procedure and other paths are used for the retrieval tests. Each path started with the entrance (E), contained three decision boxes, six no-decision boxes, and finished with the reward box (R).

tests, several different paths are used. During a test, only one bee is allowed into the maze at a time and is tested for one of the five path configurations.

Four categories of performances are defined and one of the categories is assigned to each of them:

1. bee moves through the maze and arrives directly at the goal (reward box);
2. bee moves through the maze and arrives to the goal with one or more turns back (bee leaves the box through the hole from which it entered);
3. bee moves through the maze with mistakes (bee making one or more wrong turns at the decision boxes) but arrives to the goal;
4. bee does not arrive to the goal within 5 minutes after entering the maze.

Performances of control and treated bees are evaluated as the mean of the categories assigned to bees in each group. The time required to reach the goal from the instant of entering the maze is measured for each bee. Flight time is considered only for bees flying through the whole path within 5 minutes.

A4.1 STRENGTHS/WEAKNESSES

Au: Please note that Figure A4.1 and Figure 8.4 are the same figure; this is fine and intentional by the author.

Menzel et al. (1974) have demonstrated that honey bees in flight can associate a visual mark to a reward, and this associative learning is used by bees to negotiate a path in a complex maze (Zhang et al., 1996). After treatment with a sublethal dose of a chemical, the ability of bees to perform the task can be impaired compared

to untreated control bees (Decourtye et al., 2009). Work with this type of experimental test has indicated that orientation capacities of foragers in a complex maze can be affected by a pesticide. The maze test relies on the visual learning of foragers in relation to navigation. However, while the maze test has demonstrated effects with pesticides which are neurotoxic, there are insufficient data at this time to determine whether the test will provide useful information for chemicals with other modes of action. Additionally, bee navigation in the field relies upon several guidance mechanisms (e.g., position of sun, magnetism, etc.), unlike in the maze where performance is based on the use of a limited number of pertinent cues. Additional experiments are needed to establish whether effects on maze performance reflect what may actually occur when foragers are exposed to pesticides in the field and are confronted with complex environmental cues.

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Author: Please check the page range inserted for the reference "Menzel et al., 1974" for correctness.

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